



Vijoriya International Journal for Research & Innovation

Volume -1 — Issue -1 — [July to December] 2025 — ISSN: 3107-9806

Shelf-Life Prediction for Ethanol Extract of *Clitoria ternatea* Flower Petals using Accelerated Shelf-Life Testing Based on Arrhenius Method.

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Abstract

Clitoria ternatea, commonly known as "Nil Katarolu," is a readily available plant in Sri Lanka. The flower petals of this plant are widely used in the food and beverage industries as a natural colourant due to their vibrant blue colour. The presence of a group of anthocyanins known as ternatin is responsible for this blue colour. Other than that, the flower petal of *Clitoria ternatea* offers a good source of pharmacologically active compounds responsible for potent antioxidant, anti-diabetic, and antimicrobial activities, which include tannins, saponins, triterpenoids, flavonoids, and alkaloids. In Sri Lanka, dried flower petals are commonly used to extract the natural colourant. This drying process can degrade the bioactive compounds present in the flower petals. Therefore, the development of natural colourants using fresh *Clitoria ternatea* flower petals is important in preserving its pharmacological benefits. In this study, the shelf life of *Clitoria ternatea* fresh flower petal ethanol extract is determined using the Accelerated Shelf Life Arrhenius model. Here fresh flower petals of *Clitoria ternatea* were extracted using a 40% ethanol solution. The characteristic absorbance peaks were identified, and a comparison was made between dried and fresh flower petal extracts. Fresh flower extracts showed absorbance peaks at 620 nanometers and 574 nanometers, an indication of the presence of certain phytochemical compounds. The stability of extracts over the 32 days was checked by monitoring the change of these two peaks under different temperatures; 10°C, 28°C, and 40°C. Results indicated that the moisture content of a fresh *Clitoria ternatea* flower petal was $92.07 \pm 0.7\%$. There are similar peaks of absorbance observed for the phytochemical compounds in the extract of fresh and dried flower petals. This indicates that the same phytochemical compounds are present but that the ratio between the two characteristic absorbance peaks is highest in the fresh flower petal extract, thus a more favorable phytochemical profile. The degradation of the fresh flower petal extract over time was found to follow a first-order kinetic model. The shelf-life of the fresh flower petal ethanol extract, stored at 28 °C without any preservation, was estimated to be 34.69 days.

Keywords: *Clitoria ternatea*; natural colour; Shelf life

Cite this Article

Kavindhya, H. A. C., & Samarawickrama, D. S. (2025). Shelf-Life Prediction for Ethanol Extract of *Clitoria ternatea* Flower Petals using Accelerated Shelf-Life Testing Based on Arrhenius Method. *Vijoriya International Journal for Research & Innovation*, Volume 1(1), 94–101, July–December 2025. <https://doi.org/10.65595/MDQO2737>

1 Introduction

Due to lower toxicity and health benefits, there can be seen a significant shift towards natural colours from artificial colours in today's world. Throughout history, there has been widespread evidence of the importance of natural colours in various areas, including food, textiles, art, and cultural applications. In the 19th century, with the industrial revolution, people used artificial colour compounds because they offered a vibrant, consistent, and affordable alternative to natural colours.¹ But the main component of synthetic colourants, azo dyes, has been associated with adverse health effects, including hyperactivity, immune system disruption, and carcinogenicity as well they cause potential impact to environmental systems.² The world is moving again to use natural colour compounds. Natural colours are derived from different sources, including plants, animals, and minerals.³ The vibrant hues in plants are produced by a range of pigments, such as carotenoids (red, orange, yellow), betalains (red, yellow), and flavonoids.⁴ Among flavonoids, anthocyanins are a class of water-soluble pigments responsible for red, blue, and purple colours, which are available in many fruits and flowers.^{4,5}

Clitoria ternatea, commonly known as Butterfly pea plant or “Nil Katarolu” in Sri Lanka, is one of the best examples of a plant that has flowers with brilliant blue anthocyanins. *Clitoria ternatea* is a perennial herbaceous plant belonging to the Fabaceae family, widely distributed in tropical Asia. Its distinctive blue colour in flower petals is given by polyacylated anthocyanins known as ternatins, which are derivatives of delphinidin 3,3',5'-triglucoside.⁶ As responsible for the colour, these ternatins are linked to a wide spectrum of pharmacological activities as well. The flower petal extract of *Clitoria ternatea* possesses significant antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and cytotoxic properties.^{7,8} This combination of natural colour and the health properties makes it a highly valuable natural resource.

In Sri Lanka, the traditional practice for obtaining blue colourant is sun drying flower petals because that method is simple and low-cost. But this sun drying process has major drawbacks because it can lead to degradation of compounds which are responsible for colour, as well as there can occur loss of water-soluble anthocyanins, flavonoids, and phenolic compounds.⁹ The use of fresh flower petals instead of dried ones has been suggested to overcome this issue. Because the extraction process is more efficient when the plant material is in its natural state with intact cellular structure and it prevents the anthocyanin profile.¹⁰ As the extraction solvent, ethanol was selected due to its effectiveness as a preservative and its ability to stabilize oxidation-sensitive compounds. A 40% ethanol solution is considered optimal for extracting *Clitoria ternatea* anthocyanins, as its moderate polarity allows for an efficient extraction of a wide range of bioactive compounds. As well, this helps to stabilize anthocyanins against degradation.¹¹

The natural colourant extracts degrade over time due to various conditions. For any natural colourant to be successfully incorporated into food and beverage products, a predicted shelf life is essential for quality control and consumer safety. Accelerated shelf-life testing (ASLT) is a method commonly used to efficiently estimate product stability. This approach subjected the product to elevated stress conditions, such as high temperature, to accelerate degradation kinetics. The relationship between the degradation rate and temperature is reliably described by the Arrhenius model, which allows for the extrapolation of shelf-life under normal storage conditions.¹² The Arrhenius equation can be given as,

$$k = k_0 \times e^{-(E_a/RT)} \quad (1)$$

(1)

where k is the quality degradation constant, k_0 constant that does not depend on temperature, E_a activation energy, T absolute temperature, and R is the gas constant. The shelf-life evaluation involves analyzing the kinetics of quality factor (Q) depletion, which may represent properties such as colour, texture, or nutrient content. In this study, the quality factor corresponds to the concentration of compounds responsible for the extract's colour. The general degradation rate is given by,

$$\frac{dQ}{dt} = k \cdot Q^n \quad (2)$$

where Q is the quality factor, t is time, k is a constant rate that depends on temperature and water activity, and n is the degree factor or reaction order. The rate of change of the quality factor, dQ/dt , can be described using different kinetic models, primarily zero-order and first-order reactions. According to previous studies, the most common degradation is first-order degradation in natural colours.^{13,14} The shelf-life of the product can be determined after finding the rate constant of reaction by using the following equations.¹⁵

Zeroth-order quality decrease reaction can be stated with the equation,

$$t = \frac{A_0 - A_t}{k} \quad (3)$$

For a first-order decrease reaction can be stated by the equation

$$t = \frac{\ln A_0 - \ln A_t}{k} \quad (4)$$

2 Methods

2.1 Plant Materials and Chemicals.

Fresh *Clitoria ternatea* flowers were collected from plants located in Panadura, Kaluthara area, from September to December 2023. The sampling was done by the hand-picking method, and only flowers with no visible symptoms were collected. The petals were separated, washed, and processed in two ways. One batch was cut into small pieces and used freshly to prepare samples, while the other batch was sun-dried, ground into fine powder, and stored in a clean airtight container until further use. By using commercially available 96.2% ethanol, prepared 40% solution was prepared for all extraction purposes.

2.2 Determination of moisture and solid content.

The moisture content of the fresh flower petals was determined by drying petals in a hot air oven at $103 \pm 2^\circ\text{C}$ for 6 hours until a constant weight was achieved. The moisture content was calculated as a percentage of weight loss compared to the initial weight. The solid content of the fresh flower petal extract was determined by oven drying a known volume of the extract and calculating the percentage of residual solids.

2.3 Extraction of colour compounds.

A solution was prepared by mixing 1.00 g of dried flower petal powder in 100 mL of 40% ethanol solvent. Another one was prepared from 8.00 g of fresh petals in 400 mL of 40% ethanol. When preparing the solution, the mixture was heated in a covered water bath for 15 minutes, allowed to cool, and then filtered. The dried petal extract was analyzed immediately, and the fresh petal extract was divided into 4 parts. One portion was analyzed on the day of preparation, and the rest was kept for stability studies.

2.4 Colourimetric analysis and Calibration.

A UV-Visible spectrophotometer was used for all colourimetric analyses. Characteristic absorbance peaks of the extract were identified as 574 nm and 620 nm by scanning from 400 nm to 700 nm. For the fresh flower petal extract, a series of dilutions was prepared to establish a Beer-Lambert calibration curve. The solid content of the fresh extract was used as a proxy for the concentration of colour compounds of the extract. (ppm) Absorbance was measured at two peak wavelengths, and the calibration plot with the highest coefficient of determination (R^2) was selected for all subsequent concentration calculations.

2.5 Accelerated Shelf-Life Testing

The stability of fresh flower petal extract was evaluated using the Accelerated Shelf-Life Testing protocol. Parts of the fresh flower extract were stored in incubators at three different temperatures (10 °C, 28 °C, and 40 °C). The absorbance of these samples was measured at each temperature on days 1, 8, 16, 24, and 32. The degradation kinetics were determined by plotting 0th order and 1st order graphs. The reaction order was determined based on the model which resulting the highest R^2 value across all temperatures. The rate constant (k) for colour degradation at each temperature was derived from the slope of the linear plot. The temperature dependence of the degradation rate was then analyzed using the Arrhenius relationship. The activation energy (E_a) was determined from the slope of the linear regression. This model was used to extrapolate the degradation rate constant (k) at a standard storage temperature of 28 °C. using $t = (\ln A_0 - \ln A_t)/k$ equation the Shelf life was determined.

3 Results & Discussion

3.1 Colourimetric Properties of Fresh vs. Dried Petal Extracts

This study investigated the use of *Clitoria ternatea* fresh flower petals instead of dried petals, which were extracted with 40% ethanol. This ethanol concentration helps stabilize anthocyanins by reducing oxidation and degradation compared to pure water or higher ethanol concentrations (ref). UV-visible spectrophotometric analysis showed that both fresh and dried extracts exhibited characteristic absorbance peaks at 574 nm and 620 nm, which are attributed to ternatins, a class of anthocyanins. The ratio of absorbance at the 620 nm peak to the 574 nm peak was higher for the fresh flower petal extract compared to the dried flower petal extract, indicating that the drying process may cause minor degradation or structural changes in anthocyanins due to exposure to

heat, light, and oxygen (ref). However, as the ratios of absorbance peaks are nearly identical, the colour difference between fresh and dried flower petals of *Clitoria ternatea* can be considered negligible. This suggests that fresh petal extract can be used as a natural colourant with colourimetric properties comparable to those of dried flower petal extract.

Table 1: Absorbance values of dried and fresh flower petal extracts

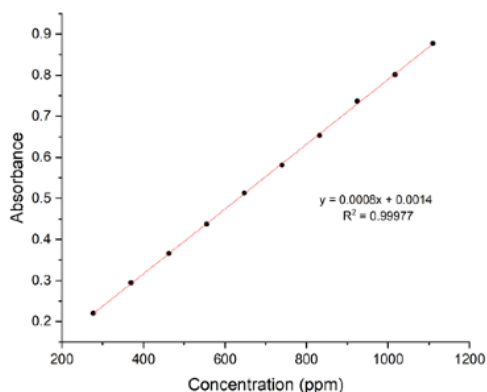
Type of flower petals	Wavelength (nm)	Absorbance	Ratio between two peaks
Dried	574	0.501	0.96
	620	0.520	
Fresh	574	0.513	0.98
	620	0.523	

3.2 Characteristic Absorbance Peak and Standard Calibration

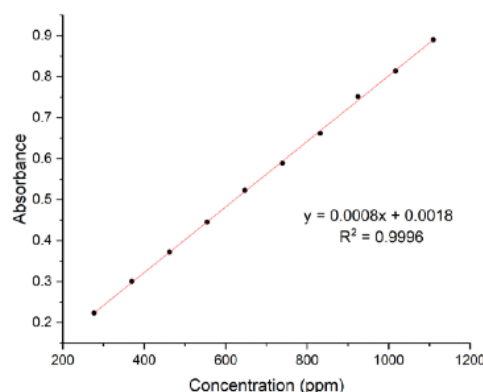
The characteristic absorption peaks for the fresh flower petal extract were observed at 620 nm and 574 nm, and these peaks remained constant across different concentrations. In this study, the concentration of colour compounds was assumed to be equivalent to the solid content of the extract, as the solid content is directly related to the amount of anthocyanins and other colour compounds present. Standard Beer–Lambert calibration plots were established at both 620 nm and 574 nm wavelengths. The calibration plot at 620 nm exhibited a superior linear fit compared to that at 574 nm. Therefore, the 620 nm peak was selected for calculating the day-by-day variation in colourant concentration.

Table 2: Linear regression equations of the standard calibration plots

Wavelength (nm)	Linear regression equation	R^2
620	$y = 0.0008x + 0.0014$	0.9998
574	$y = 0.0008x + 0.0018$	0.9996



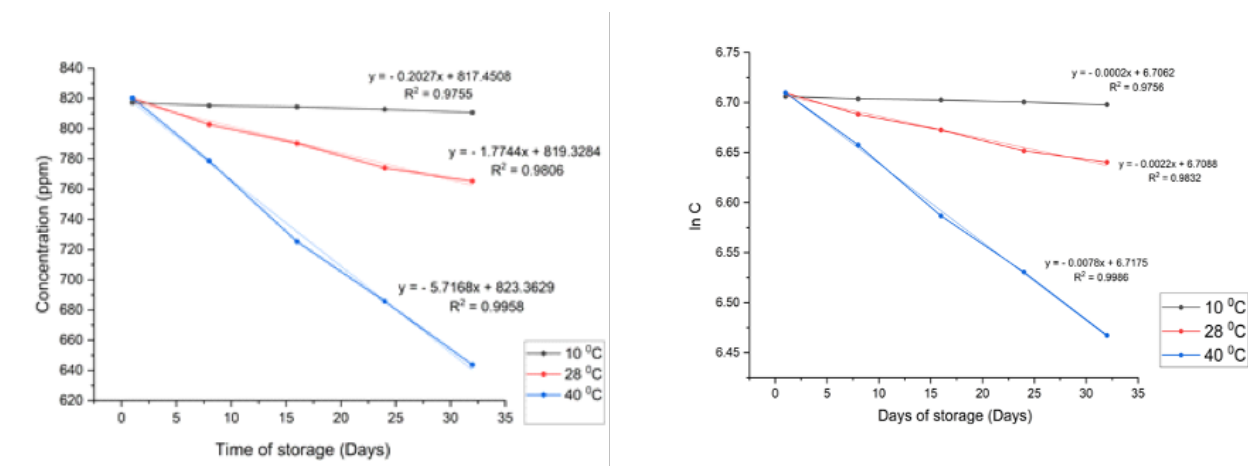
(a) Standard Beer–Lambert plot for 620 nm.



(b) Standard Beer–Lambert plot for 574 nm.

3.3 Kinetic Modelling of Colour Degradation

The degradation of colour compounds in fresh flower petal extract was monitored over time at three storage temperatures (10°C, 28°C, and 40°C). The reaction order was determined by comparing the coefficient of determination (R^2) for 0th-order and 1st-order kinetic models. According to the graphs, all temperatures at the 1st-order model yielded higher R^2 values compared to the 0th-order model. In this plot, the rate of degradation is proportional to the concentration of the colourant. Here, all the slopes were negative, which indicates a decrease in the concentration of extract. And the rate constants (k) were derived here from the slope of the first-order plots to plot the Arrhenius plot.



(a) Graphical representation of 0th-order reaction.

(b) Graphical representation of 1st-order reaction.

The determination of the Arrhenius equation is done by making the plot of $\ln k$ and $1/T$. The linear equation of the Arrhenius plot resulted as $10881.7476x + 29.9424$, and from the extrapolation, the rate constant (k) value at 28°C was obtained as 0.0020. From that, the calculated shelf life resulted as 34.69 days.

The shelf life determined is significantly influenced by storage conditions. In warmer regions of Sri Lanka, the temperature can exceed 30°C; the colour degradation is accelerated. Therefore, storage under refrigeration would be better to maintain the quality; otherwise, the shelf life would be shorter. On the other hand, in cooler regions, the extract would be more stable at ambient temperature, and the shelf life would also be high.

3.4 Shelf-Life Estimation and Storage Considerations

The determination of the Arrhenius equation is done by making the plot of $\ln k$ and $1/T$. The linear equation of the Arrhenius plot resulted as $10881.7476x + 29.9424$, and from the extrapolation, the rate constant (k) value at 28°C was obtained as 0.0020. From that, the calculated shelf life resulted as 34.69 days.

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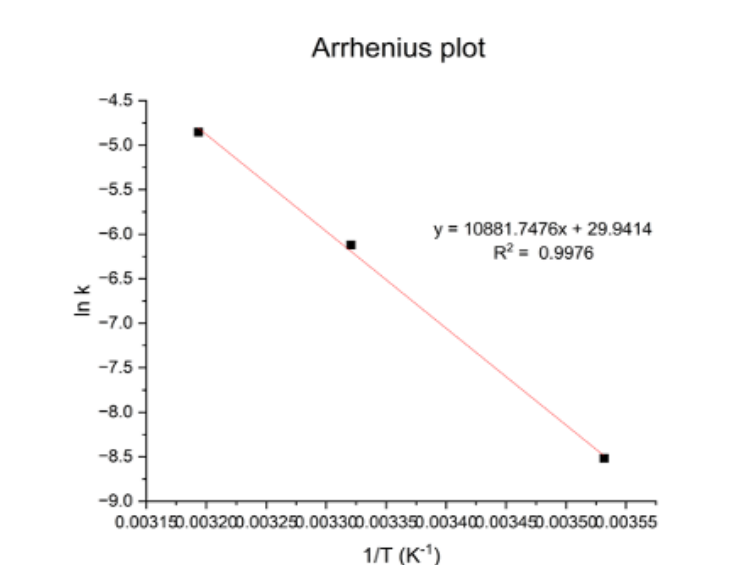


Figure 3: Arrhenius plot.

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4 Conclusion

In conclusion, the study shows both dried and fresh flower petal extracts of *Clitoria ternatea* give absorbance peaks at 620 nm and 574 nm, which is responsible for the presence of anthocyanins which give the bright blue colour of the extracts. The ratio between these two peaks was found to be higher in the fresh flower petal extract compared to the dried flower petal extract. Kinetic analysis indicated the colour degradation reaction of the fresh *Clitoria ternatea* flower petal ethanol extract is first-order. The estimated shelf life of the extract was observed as 34.69 days at 28°C and the accelerated shelf life was determined by using the Accelerated shelf-life testing – Arrhenius method.

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Cite this Article

Kavindhya, H. A. C., & Samarawickrama, D. S. (2025). Shelf-Life Prediction for Ethanol Extract of *Clitoria ternatea* Flower Petals using Accelerated Shelf-Life Testing Based on Arrhenius Method. *Vijoriya International Journal for Research & Innovation*, Volume 1(1), 94–101, July–December 2025. <https://doi.org/10.65595/MDQO2737>